Human Herpesvirus 6 Is Present in Lesions of Langerhans Cell Histiocytosis

Maureen A. Leahy, Sonja M. Krejci, Marti Friednash, Stephen S. Stockert, Harry Wilson, J. Clark Huff, William L. Weston, and Sylvia L. Brice

Department of Dermatology, University of Colorado Health Sciences Center; and the Department of Pathology, The Children's Hospital, Denver, Colorado, U.S.A.

Langerhans cell histiocytosis (LCH) is a disease characterized by Langerhans cell infiltration of skin and bone, with its most severe form manifested by multifocal infiltration of many organs. The etiology is unknown, although viral infection has been proposed as a potential pathogenic factor. Human herpesvirus 6 (HHV-6), a recently described member of the human herpesvirus family, has been associated with atypical or malignant lymphocytic processes, and immune disorders. Based on these observations, we suspected that HHV-6 may play a role in the pathogenesis of LCH.

Lesional tissue of 30 patients with LCH was retrospectively examined for the presence of HHV-6 by using the polymerase chain reaction. Tissue specimens from 63 patients with other benign and malignant histiocytic and lymphocytic diseases served as controls. In addition, all specimens were examined with control primers specific for herpes simplex virus (HSV). HHV-6 DNA was detected in lesions of 14 of 30 patients with LCH (47%). On clinical subgroup analysis, HHV-6 DNA was found in 10 of 16 patients with extraosseous disease (63%) and in four of 14 patients with disease limited to bone (29%). In each case, the prevalence of HHV-6 in LCH lesions was statistically significant, when compared to the control population. HSV DNA was not found in any of the LCH or control specimens. Although the presence of a virus alone does not establish a causal role in the disease, it supports the possibility of an etiologic relationship. From this study, we emphasize the need for further investigation of the potential HHV-6–mediated pathogenesis of LCH. Key words: polymerase chain reaction/histiocytosis X/eosinophilic granuloma/DNA. *J Invest Dermatol 101: 642–645, 1993*

Langerhans cell histiocytosis (LCH), formerly called histiocytosis X (HX), is a disease characterized by Langerhans cell infiltration of skin and bone, with its most severe form manifested by multifocal infiltration of many organs. The etiology is unknown and there is no specific therapy [1]. Immune dysfunction [2–4] infection [5–7] and neoplasia [8,9] have all been proposed as potential pathogenic mechanisms in this disease, but there is no consistent evidence for any of these factors, despite numerous investigative efforts.

Viruses have long been suspected as potential etiologic agents of LCH [6,7,10,11]. Viral infection has been proposed as a possible stimulus of poorly regulated activation of histiocytes, and an unusual host response to viral infection postulated to account for the immunologic abnormalities often seen in the disease. Viral infections have been shown to influence Langerhans cell distribution in tissues [12–14] and Langerhans cells have been reported to act as potent activators of human T cells in herpes simplex virus infection [15].

Human herpesvirus 6 (HHV-6) is a recently described member of the human herpesvirus family and is the etiologic agent of the childhood exanthem, roseola [16,17]. The seroprevalence of HHV-6 in the normal population is high, most individuals being infected as children [18–20], the population where LCH is most frequently found. HHV-6 was originally isolated from a small group of patients, all with lymphoproliferative disorders [21], and a number of subsequent studies have provided indirect evidence for a causal association of HHV-6 with atypical or malignant lymphocytic processes, and immune disorders [22–25]. The target cell for this virus appears to be primarily the T lymphocyte [26], although latent infection of monocytes/macrophages has also been reported [27].

Based on these observations, we suspected that HHV-6 may play a role in the pathogenesis of LCH. To test this hypothesis, we retrospectively examined the tissues of 30 patients with LCH for evidence of HHV-6 infection by means of the polymerase chain reaction (PCR) and compared the prevalence of the virus in LCH to that in other histiocytic and lymphocytic diseases.

MATERIALS AND METHODS

Subjects Formalin-fixed, paraffin-embedded biopsies of involved tissue (i.e., skin, bone, lung, lymph node) from 30 patients who carried the pathologic diagnosis of LCH were obtained from the Denver Children's Hospital and the University of Colorado Health Sciences Center. Light microscopy of all specimens was re-reviewed independently by both a pathologist (HW) and dermatologist (WW) to confirm the pathologic diagnosis. Both reviewers were blinded to each other's readings. The patients' medical records were reviewed to confirm the recorded biopsy site and obtain details of the clinical presentation of disease to ensure inclusion of only patients with LCH. Because LCH has protean clinical manifestations, the nomenclature used in describing its various forms can be confusing. For our study, LCH was divided into two subsets: 1) HX, defined by involvement of skin and/or multiple organ systems, including the Letterer-Siwe and Hand-Schuller-

Manuscript received March 22, 1993; accepted for publication July 7, 1993.

Reprint requests to: Dr. Sylvia L. Brice, Department of Dermatology, B-153, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262.

Abbreviations: BG, beta globin; CTCL, cutaneous T cell lymphoma; HHV-6, human herpesvirus-6; LCH, Langerhans cell histiocytosis; PCR, polymerase chain reaction; PLEVA, pityriasis lichenoides et varioliformis acuta; SCID, severe combined immunodeficiency disease.

0022-202X/93/$06.00

Table I. Clinical Profile of Cases of Extraosseous Langerhans Cell Histiocytosis (HX)

Case	Patient's Age	Sites of Involvement	Biopsy Site(s)	HHV-6 Status
I	16 years	Skin, bone, liver, lung, LNs,[a] mucosa	LN	Positive
II	1 year	Skin, bone, soft tissue, otitis	Skin	Positive
III	2 years	Skin	Skin	Positive
IV	23 years	Lung	Endobronchial	Positive
V	1 month	Skin	Skin	Positive
VI	40 years	Skin, mucosa	Skin	Positive
VII	1 year	Skin, bone, soft tissue, otitis	Soft tissue mass	Positive
VIII	2 years	Bone, soft tissue, LNs, otitis	Soft tissue mass	Positive
IX	5 years	Skin	Skin	Positive
X	1 year	Bone, soft tissue, mucosa	Soft tissue mass	Positive
XI	6 months	Skin, bone, lung, liver, spleen, LNs, bone marrow	Skin	Negative
XII	11 months	Skin, bone, mucosa, LNs, otitis	Skin, LN	Negative
XIII	5 months	Skin	Skin	Negative
XIV	2 years	Bone, soft tissue, exophthalmous, otitis, diabetes insipidus	Soft tissue mass	Negative
XV	19 months	Skin, LNs, otitis	Skin	Negative
XVI	2 years	Lung, liver, spleen, LNs	LN	Negative

[a] LN, lymph node.

Christian syndromes, and 2) eosinophilic granuloma (EG), defined by single or multiple bone lesions and no other disease manifestations. Sixteen of the tissue specimens were from patients with HX and 14 tissue specimens were bone lesions of patients with EG.

Sixty-three control biopsies of benign and malignant histiocytic and lymphocytic diseases of the skin were obtained from the same institutions' pathology departments. Three tissue specimens were obtained from the lesional skin of patients with congenital self-healing reticulohistiocytosis (CSHR), one from lesional skin of a patient with benign cephalic histiocytosis (BCH), nine from lymphomatoid papulosis, ten from pityriasis lichenoides et varioliformis acuta (PLEVA), 38 from cutaneous T-cell lymphoma (CTCL), one from the lymph node of a patient with severe combined immune deficiency disease (SCID), and several skin and lymph node biopsies from a patient with SCID with reticuloendotheliosis and eosinophilia (Omenn's disease).

Extraction of DNA Thirty 5-μm sections were cut from each block and placed into sterile tubes. The sections were deparaffinized with xylene and 100% ethanol and incubated overnight at 65°C in 10 μl Proteinase K (10 mg/ml; BRL, Gaithersburg, MD) and 90 μl lysis buffer (10 mM Tris, 1 mM ethylenediamine tetraacetic acid (EDTA), 0.1% sodium dodecylsulfate). DNA was then isolated by two successive phenol extractions, precipitated in ethanol, resuspended in 100 μl sterile TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and stored at −20°C until studied.

DNA Amplification Amplification of DNA was accomplished using the polymerase chain reaction (PCR). The HHV-6 primers used were 25-base-pair oligonucleotides that amplify a 223-base-pair portion of the 13R open reading frame of the HHV-6 strain U1102 genome [28]. These have been previously shown to be specific for HHV-6 [29]. Primers that amplify a 110-base-pair portion of the human beta-globin (BG) gene were used to verify the quality of DNA extracted from each specimen, as previously described [30]. HHV-6-positive control DNA was obtained by standard phenol extraction of HHV-6 strain GS-infected HSB-2 cells [31]. Negative control DNA was extracted from uninfected HSB-2 cells. Five microliters of each sample were added to 25 μl of reaction mixture containing 0.6 units of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT), prepared according to the recommendations of the Cetus Corporation. The thermocycling procedure consisted of initial denaturation at 94°C for 12 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1.5 min, and extension at 72°C for 1.5 min, followed by a final extension at 72°C for 10 min.

As an additional control, all specimens were also examined for the presence of HSV DNA using the PCR [32]. The primers used have sequence homology within both the HSV-1 and HSV-2 DNA polymerase genes and the predicted amplified product was 224 base pairs. The PCR protocol used to amplify HSV DNA was identical to that described for amplification of HHV-6 DNA.

DNA Detection After PCR, 15 μl of amplified product was loaded into each of the lanes of 8% polyacrylamide gels and separated electrophoretically. The DNA was then transferred to positively charged nylon membranes (Boehringer Mannheim, Indianapolis, IN). Positive and negative DNA controls were included on each gel, to ensure that the amplification was efficient and that no contamination had occurred. The membranes were hybridized at 50°C overnight with HHV-6, BG, and HSV internal oligonucleotides [28,30,32] labeled nonradioactively according to the specifications of the DIG oligonucleotide 3′ end-labeling kit (Boehringer Mannheim). The membranes were washed, and bound DNA was detected by using the protocol recommended for the anti-digoxigenin alkaline phosphatase/Lumi-Phos 530 chemiluminescence system (Boehringer Mannheim).

Statistical Analysis The proportions of HHV-6 positivity in the different study groups were compared by a two-tailed Fisher exact test. All p values less than 0.05 were considered significant.

RESULTS

Clinical Profile of the Patients Sixteen patients had the diagnosis of HX. Pertinent epidemiologic and clinical data for these HX patients are shown in Table I. The mean age of patients in the HX group was 6.2 years.

Fourteen patients had the diagnosis of EG. Twelve of these 14 had unifocal or solitary EG and two had multifocal EG. The mean age of patients in the EG group was 6.3 years.

Detection of HHV-6 by PCR DNA bands of the anticipated size for BG fragments were readily detected for all patient specimens and control DNA. The amplified 223-base-pair fragment of HHV-6 DNA was detected in 14 of 30 (47%) of the total LCH specimens, including both HX and EG populations (Table II, Fig 1). The control group had only three of 63 (5%) HHV-6-positive specimens: one SCID lymph node with a histologic appearance indistinguishable from LCH and two skin biopsies, one from lymphomatoid papulosis and one from CTCL. None of the patients had evidence of HSV DNA in any specimen (data not shown).

The prevalence of HHV-6 positivity in LCH was significantly higher than that of the control population ($p < 0.001$). Ten of 16 specimens from patients with HX (63%) and four of 14 specimens from patients with EG (29%) were positive for HHV-6. When these two subpopulations of LCH were compared independently to controls, both the HX patients ($p < 0.001$) and the EG patients ($p = 0.02$) had a significantly higher prevalence of HHV-6 in affected tissues. Of note, both patients with multifocal EG were positive for HHV-6 in affected bone.

DISCUSSION

LCH has not been previously linked to a specific infectious agent, although indirect evidence supports the possibility that viral infection might be the precipitating event for the clinical, histologic, and immunologic pathology observed [6,7,11]. Viral infection has been shown to influence Langerhans cell distribution and function. Following viral vaccinations, Langerhans cells migrate from epidermis to dermis and regional lymph nodes [14] and increased Langerhans cells are present in the lymph nodes of patients with a variety of viral

Table II. Proportion of Cases Positive for HHV-6 DNA by Diagnosis

Diagnosis	Number Positive/Number Tested	%
LCH	14/30	47[a]
HX	10/16	63[a]
EG	4/14	29[b]
Control	3/63	5

[a] $p < 0.001$ compared to control.
[b] $p = 0.02$ compared to control.

diseases [12]. In one report, Langerhans cells were found ectopically in the liver in viral hepatitis [13]. In HIV infection, Langerhans cells can support viral replication and transmission to lymphocytes [33,34]. Langerhans cells have been shown to act as potent activators of human T cells in herpes simplex virus infection [15]. Hence, the proliferation of Langerhans cells in LCH may be a physiologically appropriate, but clinically pathologic, response to a viral infection.

The immune abnormalities seen in LCH could also be explained by viral infection. The most consistent abnormalities seen have been a decrease in number and function of suppressor T cells and dysmorphic changes in the thymus, although monocyte dysfunction and B-cell abnormalities, including elevated antibody levels, have also been reported. HIV is well known to stimulate CD8+ cells and to cause persistent antibody production; now there is evidence that HIV infection of dendritic cells may underlie both effects [35]. In LCH, a different virus might activate histiocytes and also impair immune regulation of subsequent histiocytic proliferation, by disabling the suppressor T cell and pathologically amplifying the host immune response to the primary infection. Dysmorphic thymic changes seen in LCH are similar to those seen in a patient with congenital rubella [36] and in patients with HIV infection [37,38] suggesting that thymic and T-cell dysfunction could be a direct effect of viral infection. Because HHV-6, a lymphotropic virus specific for T cells [21–26], also latently infects human monocytes/macrophages [27], it would be a prime candidate agent in the initiation of LCH.

Other histiocytic diseases have been linked to viral infection. Risdall *et al* studied 19 patients with bone marrow histiocytosis accompanied by prominent erythrophagocytosis and found 14 patients with active HSV infections, one with adenovirus, and one with Epstein-Barr virus [39]. All patients had absence of response to mitogens, which normalized upon recovery. Most recently, Levine *et al* studied nine patients with sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease), a disease of histiocytic origin usually presenting as painless cervical lymphadenopathy in children and young adults. Using *in situ* hybridization, the investigators were able to detect HHV-6 DNA in seven of nine cases studied and noted that the HHV-6 sequences tended to be localized in lymph node sinuses that contained abnormal histiocytes [40]. *In situ* hybridization studies of LCH and control tissue might localize the HHV-6 DNA to a particular cell (e.g., the Langerhans cell) and help to clarify the potential pathogenic role of the virus in this disease.

In summary, HHV-6 DNA was demonstrated in the involved tissue of 14 of 30 patients with LCH and in an even higher percentage of cases of multisystem HX. These results were highly significant when compared to those obtained from the control population of benign and malignant histiocytic and lymphocytic diseases. Although presence of a virus alone does not establish a causal role in any given disease, it certainly supports the possibility of an etiologic relationship. From this study, we emphasize the need for further investigation of the potential HHV-6 mediated pathogenesis of LCH, and also the impact of HHV-6 infection on immune regulation in the human host.

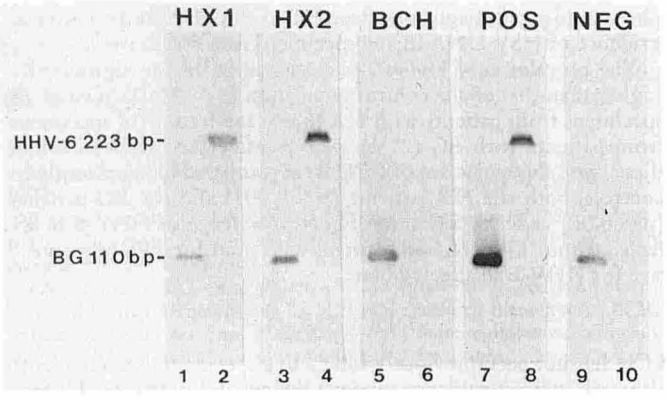

Figure 1. HHV-6 detection by PCR. Autoradiograph obtained after hybridization of labeled probes with DNA transferred to nylon membranes, demonstrating the presence of both BG and HHV-6 DNA in the lesions of two different patients with HX *(lanes 1–4)* and the absence of HHV-6 DNA in a lesion of BCH *(lanes 5 and 6)*. Positive and negative control DNA is demonstrated in *lanes 7–10*.

We are grateful to Philip E. Pellett, of the Center for Disease Control in Atlanta, for providing us with HHV-6 DNA.

The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HSB-2 from Electro-Nucleonics, Inc. and HSB-2/HHV-6 from Dr. Robert Gallo.

REFERENCES

1. Osband ME: Langerhans' cell histiocytosis. *Hematol Oncol Clin North Am* 1:737–751, 1987
2. Leiken SL: Immunobiology of histiocytosis X. *Hematol Oncol Clin North Am* 1:49–61, 1987
3. Osband ME, Lipton JM, Lavin P, *et al:* Histiocytosis X: demonstration of abnormal immunity, T-cell histamine H2-receptor deficiency, and successful treatment with thymic extract. *N Engl J Med* 304:146–153, 1981
4. Hamoudi AB, Newton WA, Mancer K, Penn GM: Thymic changes in histiocytosis. *Am J Clin Pathol* 77:169–173, 1982
5. Hand A: Defects of membranous bones, exophthalmos and polyuria in childhood: is it dyspituitarism? *Am J Med Sci* 162:509–515, 1921
6. Favara BE: Langerhans' cell histiocytosis pathobiology and pathogenesis. *Semin Oncol* 18:3–7, 1991
7. Nezelof C, Basset F, Rousseau MF: Histiocytosis X: histiogenetic arguments for a Langerhans' cell origin. *Biomedicine* 118:365–371, 1973
8. Ornvold K, Carstensen H, Larsen JK, Christensen IJ, Ralfkiaer E: Flow cytometric DNA analysis of lesions from 18 children with Langerhans' cell histiocytosis (histiocytosis X). *Am J Pathol* 136:1301–1307, 1990
9. Abdelatif OMA, Chandler FW, Pantazis CG, McGuire BS: Enhanced expression of c-myc and H-ras oncogenes in Letterer-Siwe disease. *Arch Pathol Lab Med* 114:1254–1260, 1990
10. Favara BE, McCarthy RC, Micrau GW: Histiocytosis X. *Human Pathol* 14:663–676, 1983
11. Basset F, Soler P, Hance AJ: The Langerhans' cell in human pathology. *Ann NY Acad Sci* 465:324–339, 1986
12. Vernon ML, Fountain L, Krebs HM, Horta-Barbosa L, Fuccillo DA, Sever JL: Birbeck granules (Langerhans' cell granules) in human lymph nodes. *Am J Clin Pathol* 60:771–779, 1973
13. Scotto J. Cellules de Langerhans dans le foie humain. *Nouv Presse Med* 6:3864, 1977
14. Nagao S, Inaba S, Iijima S: Langerhans' cells at the sites of vaccinia virus inoculation. *Arch Dermatol Res* 256:23–31, 1976
15. Braathen LR, Berle E, Mobech-Hanssen U, Thorsby E: Studies on human epidermal Langerhans' cells. II. Activation of human T lymphocytes to herpes simplex virus. *Acta Derm Venereol* 60:381–387, 1980
16. Yamanishi K, Okuno T, Shiraki K, *et al:* Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* I:1065–1067, 1988
17. Asano Y, Nakashima T, Yoshikawa T, Suga S, Yazaki T: Severity of human herpesvirus-6 viremia and clinical findings in infants with exanthem subitum. *J Pediatr* 118:891–895, 1991
18. Briggs M, Fox J, Tedder RS: Age prevalence of antibody to human herpesvirus 6. Lancet I:1058–1059, 1988
19. Brown NA, Sumaya CV, Liu C-R, *et al:* Fall in human herpesvirus 6 seropositivity with age. *Lancet* II:396, 1988
20. Pruksananonda P, Hall CB, Insel RA, *et al:* Primary human herpesvirus 6 infection in young children. *N Engl J Med* 326:1445–1450, 1992

21. Salahuddin SZ, Ablashi DV, Markham PD, *et al:* Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 234:596–601, 1986
22. Krueger GRF, Koch B, Ramon A, *et al:* Antibody prevalence to HBLV (HHV-6) and suggestive pathogenicity in the general population and in patients with immune deficiency syndromes. *J Virol Methods* 1:1058–1059, 1988
23. Downing RG, Sewankambo N, Serwadda D, *et al:* Isolation of human lymphotropic herpesviruses from Uganda. *Lancet* II:390, 1987
24. Jarrett RF, Gledhill S, Qureshi F, *et al:* Identification of human herpesvirus-6-specific DNA sequences in two patients with non-Hodgkin's lymphoma. *Leukemia* 2:496–502, 1988
25. Agut H, Guetard D, Collandre H, *et al:* Concomitant infection by human herpesvirus 6, HTLV-1, and HIV-2. Lancet I:712, 1988
26. Takahashi K, Sonoda S, Higashi K, *et al:* Predominant CD4 T-lymphocyte tropism of human herpesvirus 6-related virus. *J Virol* 63:3161–3163, 1989
27. Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K: Latent human herpesvirus-6 infection of human monocytes/macrophages. *J Gen Virol* 72: 1401–1408, 1991
28. Lawrence GL, Chee M, Craxton MA, Gompels UA, *et al:* Human herpesvirus 6 is closely related to human cytomegalovirus. *J Virol* 64:287–299, 1990
29. Gopal MR, Thomson BJ, Fox J, *et al:* Detection by PCR of HHV-6 and EBV DNA in blood and oropharynx of healthy adults and HIV-seropositives. *Lancet* 335:1598–1599, 1990
30. Kogan SC, Doherty M, Gitschier J: An improved method of prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. *N Engl J Med* 317:985–990, 1987
31. Salahuddin SZ, Ablashi DV, Markham PD, *et al:* Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 234:596–601, 1986
32. Yamamoto LJ, Tedder DG, Ashley R, Levin MJ: Herpes simplex virus type 1 DNA in cerebrospinal fluid of a patient with Mollaret's meningitis. *N Engl J Med* 325:1082–1085, 1991
33. Berger R, Gartner S, Rappersberger K, *et al:* Isolation of human immunodeficiency virus type 1 from human epidermis: virus replication and transmission studies (abstr). *J Invest Dermatol* 99:111S, 1992
34. Dusserre N, Dezutter-Dambuyant C, Mallet F, *et al:* The *in vitro* HIV-1 entry and replication in Langerhans cells may clarify the HIV-1 genome detection by PCR in epidermis of seropositive patients. *J Invest Dermatol* 99:99S–102S, 1992
35. Knight SC, Patterson S, Macatonia SE: Stimulatory and suppressive effects of infection of dendritic cells with HIV-1. *Immunol Lett* 30:213–218, 1991
36. Garcia AGP, Olinto F, Fortes TGO: Thymic hypoplasia due to congenital rubella. *Arch Dis Child* 49:181–185, 1974
37. Grody W, Fligiel S, Naeim F: Thymus involution in the acquired immunodeficiency syndrome. *Am J Clin Pathol* 84:85–95, 1985
38. Joshi VV, Oleske JM, Saad S, *et al:* Thymus biopsy in children with acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 110:837–842, 1986
39. Risdall RJ, McKenna RW, Nesbit ME, *et al:* Virus-associated hemophagocytic syndromes. A benign histiocytic proliferation distinct from malignant histiocytosis. *Cancer* 44:993–1002, 1979
40. Levine PH, Jahan N, Murari P, Manak M, Jaffe ES: Detection of human herpesvirus 6 in tissues involved by sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). *J Infect Dis* 166:291–295, 1992